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Blood cultures

Bacteraemia in critically ill patients has a mortality rate of 26%, but clinical parameters are often not reliable predictors of this condition.[1] Intravenous catheter-related infection is a common source of bacteraemia in these patients.

The correct approach to specimen collection is therefore extremely important when obtaining blood culture specimens. Great care must be taken to prevent contamination when managing patients and instituting therapy. Thorough skin disinfection and a strict aseptic technique are essential.

In 1991 Bates *et al.*[2] reported that the average inpatient with a contaminated blood culture specimen accumulated US$4 385 in excess charges and stayed in hospital 4.5 additional days compared with a similar patient without a contaminated blood culture specimen.

In 1998 the average blood culture contamination rate in published data from 155 institutions was 2.94%.[3]

Indications

Indications for blood culture vary and are not standardised. Fever is regarded as the most important indication, but older patients may be afebrile during a bacteraemic episode. Hypotension is another indication, especially if there is no other explanation for the low blood pressure.

It is generally not necessary to repeat blood cultures after therapy has commenced, but patients with fungaemia and bacteraemia caused by *Staphylococcus aureus* should be subjected to repeated cultures to document clearing of the infection. Continuing infection of the blood with these organisms may indicate deep or endovascular infection requiring surgery.

Timing and intervals

Collect blood for culture *before* initiating antimicrobial therapy. If empirical treatment is an emergency, draw the blood immediately before administering the

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next dose of antibiotic, but this is not ideal. There are no data suggesting that sampling for culture purposes coinciding with the appearance of fever or chills will maximise the yield. Similar yields are obtained when samples are collected simultaneously within 2 hours or within 24 hours of the initial blood culture.

Number of sets

Weinstein *et al.*[4] established that 91.5% and 99.3% of all bacteraemic episodes could be identified using a single culture set and two sets, respectively. An additional advantage of two sets of cultures is that skin colonisers causing bacteraemia can be excluded if organisms are not present in both sets. Three sets are recommended in cases of constant bacteraemia, e.g. endocarditis, and more sets may be needed if the causative organism, e.g. *S. epidermidis*, is a possible contaminant of prosthetic valve endocarditis.

If unusual organisms are considered as a cause of the bacteraemia, e.g. the group of organisms that cause 'culture negative' endocarditis, or brucellosis, it must be indicated on the laboratory request form as it might influence the incubation time of the culture.

Collection

Blood should be obtained from peripheral venous or arterial sites. Contamination of culture specimens is significantly increased if blood is drawn through intravenous catheters, arterial lines or inguinal vessels. If it becomes necessary to obtain culture samples from these catheters, an effort should be made to draw a second set of blood culture specimens from a peripheral venepuncture. Blood culture samples should be labelled to reflect the site from which the blood was collected as this facilitates interpretation.

Skin preparation

Each set of cultures should preferably be taken from a different area. Attention to aseptic skin cleansing is vital if blood

culture contamination is to be minimised. The skin should be prepared with 70% isopropyl alcohol, followed by 2% iodine tincture, or 0.5% alcoholic chlorhexidine. Do not use iodine compounds and chlorhexidine simultaneously as the two agents are antagonists.

Volume

The volume of blood to be cultured is still regarded as critical in obtaining an optimal result.[5] The number of organisms in the blood during a bacteraemic episode is low. Most authors recommend 20 - 30 ml blood per culture set (or 10 - 15 ml per bottle). Drawing more than 30 ml of blood per culture set does little to improve the sensitivity of the blood culture. The recommended volume for neonates is 1 - 2 ml per set, for babies 2 - 3 ml per set, and for children 3 - 5 ml per set.

References

1. Pittet D, Tarara D, Wenzel RP. Nosocomial bloodstream infection in critically ill patients: Excess length of stay, extra costs and attributable mortality. *JAMA* 1994; 271: 1598-1601.
2. Bates DW, Goldman L, Lee TH. Contaminant blood cultures and resource utilization: the true consequences of false-positive results. *JAMA* 1991; 265: 365-369.
3. Schifman RB, Strand CL, Meier FA, Howanitz PJ. Blood culture contamination: a College of American Pathologists Q-Probes study involving 640 institutions and 497 134 specimens from adult patients. *Arch Pathol Lab Med* 1998; 122: 216-221.
4. Weinstein MP, Reller LB, Murphy JR, *et al.* The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults; laboratory and epidemiologic observations. *Rev Infect Dis* 1983; 5: 35-53.
5. Bouza E, Sousa D, Rodriguez-Creixems M, *et al.* Is the volume of blood cultured still a significant factor in the diagnosis of bloodstream infections. *J Clin Micro* 2007; 45(9): 2765-2769.

C-reactive protein and procalcitonin as markers of infection

More than 40% of patients in intensive care units in Europe or Australasia have sepsis or severe sepsis, but only 58% of clinically suspected infections are confirmed by positive culture.[1] The decision to stop or continue antimicrobial treatment is

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The interpretation of Streptococcus pneumoniae antimicrobial susceptibilities

arbitrary and to some extent relies on the measurement of inflammatory markers.

Although several inflammatory markers, including interleukins, neopterin and tumour necrosis factor-alpha, are available, C-reactive protein (CRP) and procalcitonin (PCT) are most commonly used.

CRP

CRP is an acute-phase protein produced by the liver. During an inflammatory process the CRP level can rise to above normal limits within 6 hours, peaking at about 48 hours. Because the half-life of CRP is constant, levels are mainly determined by the rate of production and therefore by the severity of the precipitating cause. Even though it is a nonspecific marker, measuring CRP values can prove useful in determining disease progress or the effectiveness of treatment.

A recent study[2] of the usefulness of consecutive CRP measurements in following up cases of severe community-acquired pneumonia found that delayed normalisation of CRP in the first 3 - 7 days was suggestive of inappropriate empirical antimicrobial therapy. Patients with a decline of <60% in CRP levels in 3 days or of <90% in 7 days had a 4 - 7-fold increased risk of having received inappropriate antimicrobial treatment.

PCT

PCT is a precursor of calcitonin and is produced by the C-cells of the thyroid gland. PCT normally has a plasma level of <0.1 µg/l in healthy people, but levels rise in response to triggers such as endotoxin and inflammatory cytokines. Such levels will rise within 4 hours of endotoxin challenge and peak at 8 - 24 hours. It is a stable marker that reflects severity of disease, and with a half-life of about 24 hours it allows for repeated measurements that may reflect response to therapy.

One of the advantages of using PCT as a marker for systemic infection is the high negative predictive value (almost 99%). A negative result therefore implies that infection is unlikely and antimicrobial therapy unnecessary, thus reducing inappropriate antimicrobial usage.[3]

PCT has also been shown to have a high specificity for the diagnosis of systemic infection, using a cut-off value of 0.5 µg/l, but it is important to perform serial measurements. A rising value should prompt a review of antimicrobial treatment or source control.

It is however important to note that PCT is not a gold standard for the diagnosis of sepsis. It shows no response in localised infection where there is no systemic involvement, viral infection, fungal infection and infection with intracellular organisms. Major surgery and patients with burns may have a high baseline PCT value. The best threshold value in critically ill patients still needs to be defined and the threshold value of semi-quantitative kits may be too high to exclude systemic infection.

The PCT assay must preferably be quantitative, and daily serial measurements should be done to monitor progress over time.

References

1. French C. The epidemiology of sepsis – is Australasia different? *Critical Care and Resuscitation* 2006; 8: 219-222.
2. Bruns AHW, Oosterheert JJ, Hak E, *et al.* Usefulness of consecutive CRP measurements in follow up of severe community acquired pneumonia. *Eur Respir J* 2008; 32: 726-732.
3. Shehabi Y, Seppelt I. Pro/con debate: is procalcitonin useful for guiding antibiotic decision making in critically ill patients? *Crit Care* 2008; 12: 211.

Streptococcus pneumoniae is a common cause of respiratory tract infection. It is the most common bacterial cause of community-acquired pneumonia, sinusitis and otitis media. The emergence of pneumococcal resistance to penicillin and to other commonly prescribed antimicrobials is of specific concern globally and in South Africa. During the last 6 months of 2007, upper respiratory tract isolates' susceptibility to penicillin in South Africa varied between 27% and 60% geographically, while the susceptibility of lower respiratory tract isolates varied between 54% and 93%.[1]

Pneumococcal resistance to macrolides is another problem, and invasive *S. pneumoniae* resistant to levofloxacin has recently been described as emerging in South Africa in children receiving quinolones as part of multidrug-resistant tuberculosis therapy.[2]

To complicate matters, in 2008 the Clinical Laboratory Standards Institute (CLSI) revised the guidelines for pneumococcal reporting according to site of infection, as summarised in Table I.[3]

Reporting

Penicillin and parenteral third- and fourth-generation cephalosporins are reported according to whether the site of infection is meningeal or non-meningeal.

Table I. Clinical Laboratory Standards Institute 2008, breakpoints for reporting *S. pneumoniae*

	Susceptible	Intermediate	Resistant	Treatment advice
Penicillin				
IV non-meningitis	≤2 µg/ml	4	≥8	
IV meningitis	≤0.06	-	≥0.12	Maximum doses should be given
Oral pen V	≤0.06	0.12 - 1	≥2	
Amoxicillin Non-meningitis	≤2 µg/ml	4	≥8	
Ceftriaxone Non-meningitis	≤1	2	≥4	
Meningitis	≤0.5	1	≥2	Maximum doses should be given
Cefepime Non-meningitis	≤1	2	≥4	
Meningitis	≤0.5	1	≥2	

Laboratory confirmation of the diagnosis is difficult and colonising flora may hamper the detection of true pathogens.

Most laboratories will report susceptibilities of *S. pneumoniae* according to the minimum inhibitory concentration (MIC) to both penicillin and ceftriaxone. The values obtained will then be interpreted according to whether the site of infection is meningeal, or another site, including inner ear (otitis media), sinus (sinusitis), or lung (pneumonia). In severe pneumococcal otitis media or sinusitis it may be prudent to consult a microbiologist, as current breakpoints may not reflect optimal pharmacodynamics or kinetics for these infections. It is also important to provide adequate clinical information on the pathology request form to enable interpretation of pneumococcal susceptibilities. In practice a distinction will be made between oral and parenteral therapy with the following comments as examples:

Parenteral treatment

All pneumococcal infections (excluding meningitis) with a penicillin MIC of ≤2 µg/ml can be treated with IV penicillin 12 million units per day. These isolates can also be considered susceptible to amoxicillin and ceftriaxone. Strains with an intermediate penicillin MIC of 4 µg/ml may require 18 - 24 million units per day.

Oral treatment

All pneumococcal isolates (excluding meningitis) with a penicillin MIC of ≤0.06 µg/ml can be considered susceptible to amoxicillin, amoxicillin-clavulanic acid, cefaclor, loracarbef, cefprozil, cefuroxime and cefpodoxime for approved indications. Isolates with an MIC of 0.12 - 1 µg/ml should be treated with an increased dosage of amoxicillin or amoxicillin-clavulanic acid, or a different class of antibiotic to which the isolate is susceptible.

References

1. Senekal M. Optimizing antimicrobial therapy for the treatment of respiratory tract infection. *Modern Medicine* 2008; 33(5): 42-46.
2. Von Gottberg A, Klugman KP, Cohen C, *et al.* Emergence of levofloxacin non-susceptible Streptococcus pneumoniae and treatment for multi-drug resistant tuberculosis in children in South Africa: a cohort observational surveillance study. *Lancet* 2008; 371: 1108-1113.
3. Clinical Laboratory Standards Institute 2008 M100-S18; 28(1): 126-128.

Laboratory diagnosis of paediatric lower respiratory tract infection

The burden of respiratory tract infections (RTIs) in children is extremely high in industrialised and developing countries, with nearly 50% of paediatric consultations in industrialised countries related to RTIs.[1]

Viruses have been shown to cause up to 90% of pneumonias, especially in the first year of life, with the respiratory syncytial virus the most important pathogen. This percentage decreases to approximately 50% by school-going age. Viral pneumonias are frequently complicated by bacterial infections, and mixed infections are common. *Streptococcus pneumoniae* is common in all age groups, and complications are usually associated with pneumococcal infections or co-infections.

The majority of patients with viral lower respiratory tract infections (LRTIs) are admitted to hospital because of bronchiolitis and pneumonia.

Laboratory diagnosis

Laboratory confirmation of the diagnosis is difficult and colonising flora may hamper the detection of true pathogens.

Although respiratory virus infections cause significant morbidity and mortality, laboratory diagnosis may not be needed in all patients or under all conditions. The indications for laboratory diagnosis of viral LRTIs are not clearly defined. However, infections that lead to hospital admission or prolong hospital stay, such as moderate to severe LRTI or fever syndrome, especially in young infants or immunocompromised children, warrant laboratory investigation. Other possible indications include an effort to stop unnecessary prescription of antimicrobials, to decrease further unnecessary diagnostic testing, to direct antiviral therapy or to define seasonal or epidemic disease in a specific community.[2]

In the past tissue culture techniques, which were the gold standard, were used to detect respiratory viruses. However, these techniques are time consuming, with decreased sensitivity, compared with molecular testing that is only performed in a few specialised laboratories. Serology is rarely helpful during acute disease owing to the possible time delay before seroconversion. Several antigen-based rapid diagnostic assays are available, including immunofluorescence and ELISA assays, but nucleic acid amplification tests such as the multiplex polymerase chain reaction (PCR) offer a broad range of diagnostic capabilities where multiple aetiologies can be detected. These can include the detection of influenza virus A, B and C, adenovirus, metapneumovirus, RSV A and B, rhinovirus A, parainfluenza virus 1, 2 and 3, echovirus, bocavirus and coronavirus in a single test.

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A range of samples, such as nasopharyngeal aspirates or swabs and bronchoalveolar lavage specimens, are appropriate for PCR processing. Nasopharyngeal swabs present an easy method of obtaining a specimen if other respiratory samples are difficult to obtain. Samples should preferably not be frozen or stored for more than 72 hours before processing.

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Confirmation of bacterial aetiology can be performed by conventional culture of the organism or by antigen detection from a urine sample, e.g. in the case of *Legionella* or *S. pneumoniae*. Several PCR assays are available for the detection of typical and atypical bacterial agents.

An adequate, expectorated sputum specimen should contain many neutrophils and few or no squamous epithelial cells. The latter are indicative of contamination with saliva. If possible, patients should brush their teeth and/or rinse their mouths well with water before attempting to collect the specimen in order to reduce the possibility of contaminating it with food particles or oropharyngeal secretions. An induced sputum sample should be considered if expectorated sputum cannot be obtained.

In bronchoscopy and aspirated specimens reduction of contamination may be accomplished by a head-down position to reduce gravitational flow of saliva. Oral contamination may successfully be reduced by using a telescoping double catheter with a plug to protect the brush or by a sheathed brush. The use of the telescoping plugged catheter and bronchoalveolar lavage increases the overall diagnostic yield.

A processing delay of more than a few hours may result in loss of recovery of fastidious pathogens and overgrowth of oropharyngeal flora.

References

1. Schaad UB. Prevention of paediatric respiratory tract infections: emphasis on the role of OM-85. *Eur Respir Rev* 2005; 14: 74-77.
2. Henrickson KJ. Advances in the laboratory diagnosis of viral respiratory disease. *Pediatr Infect Dis J* 2004; 23(1S): S6-S10.

Single Suture

Cardiac causes of death after stroke

Death from cardiac causes is common in people who have survived a stroke. Part of the explanation is co-existing coronary artery disease. However, Fred Rincon and colleagues, using data from the Northern Manhattan Study, think that brain injury could contribute directly to cardiac dysfunction – perhaps through autonomic pathways.

They found that 7% of people who had a stroke had a fatal cardiac event during 4 years of follow-up – mainly acute myocardial infarction or sudden cardiac death. Those whose stroke affected the left parietal lobe were particularly at risk. However, autonomic function was not measured, so it is not known whether or not this is the underlying mechanism.

Stroke 2008; 39: 2425.

Single Suture

Cheap 'polypill' on the cards

Tests of a single, cheap tablet combining a range of drugs that protect against heart diseases and stroke have begun. The idea was first put forward years ago to decrease deaths from the big killer diseases, but pharmaceutical companies were reluctant to take on the project because cheaper drugs don't offer a financial incentive.

However, a team funded by the Wellcome Trust in London and the British Heart Foundation, led by Anthony Rodgers at the University of Aukland, New Zealand, has begun recruiting volunteers in 6 countries for a trial of a polypill to be manufactured in India. The Red Heart Pill, costing $1 for a month's supply, blends aspirin, a statin, an ACE inhibitor and a thiazide. Large-scale trials could start next year.

The polypill is aimed at both the rich and the poor, but is most likely to be used in poor countries – with the pill being offered 'blind' to almost everyone over the age of 55 in the developing world. However, people who have good access to doctors and to drugs are likely to continue to receive tailored treatment – possibly with different polypills containing varying doses of different drugs.

New Scientist 2008; 4 October: 6.